

EPA-PNL-4981

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02/04/2010 12:38 PM

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cc Kristina Ramstad  
bcc  
Subject Sockeye publication

1 attachment



Ramstad et al. 2010 (LAmS).pdf

Hello All,  
Attached you will find the latest greatest installment from Dr. Ramstad's Ph.D. research on sockeye salmon evolutionary ecology.  
Kristina now lives in New Zealand and is working on little spotted Kiwi genetics and conservation. Her contact information is:

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## Recent local adaptation of sockeye salmon to glacial spawning habitats

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Received: 16 April 2008 / Accepted: 19 June 2009 / Published online: 16 July 2009  
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**Abstract** Salmonids spawn in highly diverse habitats, exhibit strong genetic population structuring, and can quickly colonize newly created habitats with few founders. Spawning traits often differ among populations, but it is largely unknown if these differences are adaptive or due to genetic drift. To test if sockeye salmon (*Oncorhynchus nerka*) populations are adapted to glacial, beach, and tributary spawning habitats, we examined variation in heritable phenotypic traits associated with spawning in 13 populations of wild sockeye salmon in Lake Clark, Alaska. These populations were commonly founded between 100 and 400 hundred sockeye salmon generations ago and exhibit low genetic divergence at 11 microsatellite loci ( $F_{ST} < 0.024$ ) that is uncorrelated with spawning habitat type. We found that mean  $P_{ST}$  (phenotypic divergence among populations) exceeded neutral  $F_{ST}$  for most phenotypic traits measured, indicating that phenotypic differences among populations could not be explained by genetic drift alone. Phenotypic divergence among populations was associated with spawning habitat differences, but not with neutral genetic divergence. For example, female body color was lighter and egg color was darker in glacial than non-glacial habitats. This may be due to reduced sexual selection for red spawning color in glacial habitats and an apparent trade-off in carotenoid allocation to body and egg color in females. Phenotypic plasticity is an unlikely source of phenotypic differences because Lake Clark sockeye salmon spend nearly all their lives in a common environment. Our data suggest that Lake Clark sockeye salmon populations are adapted to spawning in glacial, beach and tributary habitats and provide the first evidence of a glacial spawning ecotype in salmonids.

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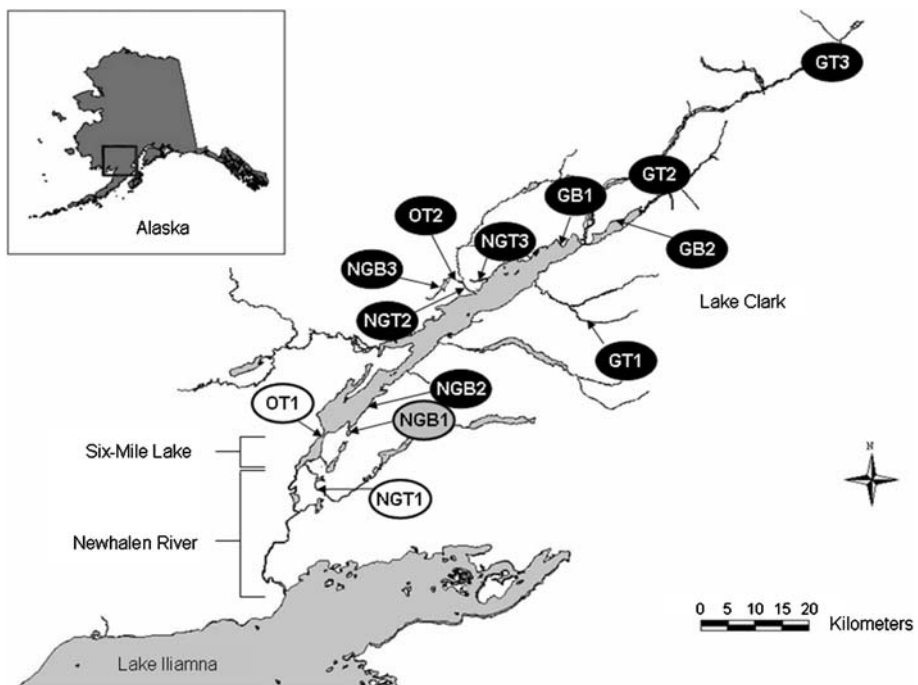
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Glacial spawning habitats are often young (i.e., <200 years old) and ephemeral. Thus, local adaptation of sockeye salmon to glacial habitats appears to have occurred recently.

**Keywords** Local adaptation · Bottleneck · Glacial · Carotenoid · *Oncorhynchus*

## Introduction

Adaptive divergence is expected among large, relatively isolated populations experiencing different selection regimes while divergence due to drift is expected among small, isolated populations (Wright 1931; Li 1978; Allendorf 1983). It is essential to differentiate between selection and drift in promoting phenotypic divergence among populations because evolutionary inferences and management implications differ vastly between the two scenarios (Luikart et al. 1998; Soule and Mills 1998; Briskie and Mackintosh 2004). In this paper, we assess evidence of local adaptation among populations of sockeye salmon (*Oncorhynchus nerka*) spawning in glacial, beach, and tributary habitats of Lake Clark, Alaska (Fig. 1). Lake Clark sockeye salmon populations shared a common founding event approximately 100–400 hundred sockeye salmon generations ago. They exhibit low genetic divergence at 11 microsatellite loci ( $F_{ST} < 0.024$ ) that is uncorrelated with spawning habitat type (Ramstad et al. 2004). Glacial habitats in Lake Clark are geologically young and



**Fig. 1** Map of Lake Clark, Six-mile Lake, and Lake Iliamna with sampling sites shown. Sites are numbered from downstream to upstream, and coded for spawning habitat type (NGB, non-glacial beach; GB, glacial beach; NGT, non-glacial inlet tributary; GT, glacial inlet tributary; OT, outlet tributary (non-glacial)) and genetic population structure (open, Iliamna/Six-mile Lake group; grey, Sucker Bay Lake; black, Lake Clark group). Refer to Table 1 for population names

ephemeral. Thus, local adaptation to these habitats would require sockeye salmon to adapt quickly to highly unstable, geologically young habitats.

Specific natal homing and spawning habitat differences promote reproductive isolation, genetic structuring, and phenotypic divergence among populations of sockeye salmon (Quinn 1985; Quinn and Dittman 1990; Wood 1995; Ramstad et al. 2003; Hendry and Day 2005). Morphological, behavioral, and life history traits of sockeye salmon are shaped by both sexual and natural selection (see reviews in Taylor 1991; Wood 1995) and many such traits have an additive genetic basis in salmonids (e.g., morphology—Funk et al. 2005; Keeley et al. 2007, egg size—Gall and Neira 2004; but see Funk et al. 2005, carotenoid conversion and flesh color—Withler 1986; Craig and Foote 2001, age at maturity—Gall et al. 1988; Hankin et al. 1993, spawning time—Siitonen and Gall 1989; Quinn et al. 2000). Parallel patterns of phenotypic divergence between fish spawning in beach and tributary habitats among nursery lakes has prompted definition of spawning ecotypes of sockeye salmon. For example, the beach spawning ecotype is characterized by larger eggs, deeper bodies, smaller size at age, younger age at maturity, and later spawning times than the inlet tributary ecotype (Blair et al. 1993; Quinn et al. 1995, 2001; Wood 1995; Hamon and Foote 2000; Hendry et al. 2000b).

Evidence of local adaptation of salmon to glacial spawning habitats (turbidity > 5 nephelometric turbidity units (NTU) at peak spawning, Koenings et al. 1986, 1990) has never been reported. However, fish spawning in glacial and non-glacial habitats may differ phenotypically in response to inherently different selection pressures associated with these different environments. For example, fine glacial substrate (<2 mm diameter) could promote reduced egg size (Quinn et al. 1995) and reduced visibility may relax or negate sexual selection on visual stimuli (Barrett et al. 1992; Seehausen et al. 1997) in glacial spawning fish. Retreating glaciers create novel aquatic habitats that salmon have recently colonized (Kondzela and Gharrett 2007). Thus, genetic drift due to founder effects may also promote genetic and phenotypic divergence among populations spawning in glacial and non-glacial habitats.

Here we compare the effects of selection and drift in promoting phenotypic divergence among sockeye salmon populations by comparing  $P_{ST}$  (phenotypic divergence among populations, Saint-Laurent et al. 2003; Leinonen et al. 2006; Raeymaekers et al. 2007; Saether et al. 2007) and  $F_{ST}$  (neutral genetic divergence due to drift and gene flow, Wright 1951). If environmental and non-additive genetic effects on a given phenotypic trait are negligible, then  $P_{ST}$  will equal  $F_{ST}$  when divergence is due to drift and will differ significantly from  $F_{ST}$  when divergence is due to selection (Merila and Crnokrak 2001; McKay and Latta 2002; Storz 2002; Saint-Laurent et al. 2003; Leinonen et al. 2006). Phenotypic traits considered in this study are expected to meet these assumptions. They are all associated with spawning and fully developed prior to fish arriving at their spawning habitats (Hamon and Foote 2000; Hamon et al. 2000; Hendry et al. 2000a).

Lake Clark sockeye salmon spend nearly all of their lives in a common environment, thus approximating a common garden experiment in the wild. Young hatch from eggs laid in shallow gravel nests (redds) and migrate shortly after emergence to a common nursery lake, where they rear as juveniles for 1–2 years (Burgner 1991; Schlenger 1996). They then collectively migrate out to the ocean (Orrell 1963; Woolington et al. 1990) where they spend 1–4 years in a common oceanic environment (French et al. 1976; Burgner 1980). Adults return synchronously to their natal sites in Lake Clark to spawn and die (Burgner 1980; Jensen and Mathisen 1987).

The objectives of this study were to provide the first test for local adaptation of salmon to glacial spawning habitats and to re-assess evidence for local adaptation of sockeye

salmon to beach and tributary spawning habitats. Specifically we ask if phenotypic divergence among spawning populations of Lake Clark sockeye salmon is likely the result of selection or can be explained by genetic drift alone.

## Materials and methods

Large numbers of sockeye salmon spawn annually in Lake Clark (e.g., 30 thousand–8.4 million between 1979 and 1998, Rogers et al. 1999), which is one of two large lake systems in the Kvichak River watershed of Bristol Bay, Alaska (Fig. 1). Lake Clark is geologically young (12–15 thousand years old, Stilwell and Kaufman 1996) and has spawning habitats that were deglaciated as recently as 200 years ago (Heiser, personnel communication 2002). Spawning habitats in Lake Clark are highly heterogeneous (Demory et al. 1964; Brabets 2002). In this study, we formally compared fish spawning in (1) beach and inlet tributary habitats, (2) glacial and non-glacial beach habitats, and (3) glacial and non-glacial inlet tributary habitats (referred to here as tributaries). Outlet tributary populations were excluded from comparisons between habitat types because there are no glacial outlet tributaries in the Lake Clark system. However, we included two outlet tributary populations in comparisons of individual populations to maximize the spawning habitat diversity represented and neutral genetic divergence among study populations (Ramstad et al. 2004; Fig. 1).

### Spawning habitat measures

Turbidity (NTU) was measured with a Hach<sup>®</sup> Pocket Turbidimeter (Loveland, Colorado, USA) during peak spawning time at 13 spawning sites (Fig. 1). For 10 of these 13 sites, water depth (m) was measured every meter from shore and substrate size composition assessed by Wolman Pebble Count (Kondolf and Li 1992). Measures were taken along three to five randomly chosen transects crossing the wetted width of tributaries and the wetted beach shore to a depth of approximately 1.5 m directly offshore.

### Phenotypic traits

Phenotypic traits considered in this study have been shown to have moderate to high heritabilities in a number of salmonid populations and environmental settings (see 'Introduction'), a condition that is critical for meaningful comparisons of  $P_{ST}$  and  $F_{ST}$  (Saether et al. 2007). Phenotypic traits of sockeye salmon from 13 spawning populations throughout Lake Clark and Six-mile Lake, and from all spawning habitat types defined above, were measured in 2000 and 2001 (Fig. 1). Adult, spawning fish were captured on their spawning grounds by beach seine and tangle net. Morphological measurements were taken on approximately 20 fish per sex from four populations in 2000 (NGB1—Sucker Bay Lake, OT2—Little Kijik River, GB2—Little Lake Clark Beach, GT2—Lower Tlikakila River) and 30 fish per sex from 13 populations in 2001. Measurements of hypural length (HL—mid-eye to posterior edge of the hypural plate), body depth (BD—anterior insertion of the dorsal fin to belly at a 90° angle to the lateral line), and snout length (SN—tip of snout to mid-eye) were taken on the left side of each fish with calipers to the nearest mm.

Eggs of ripe and spawning females were collected from 11 to 42 fish from each of 13 populations in 2001. Eggs were immediately placed in 5% buffered formalin and stored for 63–125 days prior to processing. Salmonid eggs can be stored in this manner for at least

5 months without change in weight (Fleming and Ng 1987). Approximately 30 eggs per fish were collectively weighed to the nearest gram after removal of excess storage solution.

Body color during spawning was assessed for approximately 30 fish per sex from 13 populations in 2001. Skin color on the left lateral dorsal hump was subjectively categorized as red or pink by one of two observers. Spawning color scores were repeated both within and between observers to assess reliability of the measure. Egg color was categorized as the closest matching of plates one through five of the Hoffman-La Roche Color Card for Salmonids (Roche Vitamins and Fine Chemicals Division, Hoffman-La Roche Inc., Nutley, New Jersey, USA) by one observer with no knowledge of the body color or habitat type of the sampled females. Hoffman-La Roche color scores are highly correlated with red measures given by a Minolta CR-100 chromameter ( $a^*$ ) and carotenoid concentration (Smith et al. 1992; Craig and Foote 2001).

### Statistical analyses

We first characterized abiotic habitat differences between spawning habitat types with one-way analysis of variance (ANOVA). Substrate counts are presented as proportions by size categories that sum to one within sites, but are independent among sites (Table 1).

Body depth, snout length, and egg size were adjusted to common hypural lengths. Individual trait values were  $\log_e$  transformed and compared among populations and habitat types by analysis of covariance (ANCOVA) with  $\log_e$  HL as the covariate in SPSS<sup>®</sup> v.12.0 (SPSS, Inc., Chicago, Illinois, USA). Because body length and ocean age are highly correlated, this analysis almost completely removes the effect of both body size and age (Trippel and Hubert 1990). Traits were adjusted to the grand sample mean body size (519 mm male morphology, 500 mm female morphology, 507 mm egg size) with the following equation.

$$T_{\text{adj}} = T_{\text{obs}} \left( \frac{L_{\text{adj}}}{L_{\text{obs}}} \right)^b$$

where  $T_{\text{adj}}$  is the adjusted trait size,  $T_{\text{obs}}$  is the observed trait measure,  $L_{\text{adj}}$  is the hypural length to which the trait is being adjusted,  $L_{\text{obs}}$  is the hypural length observed, and  $b$  is the common within group slope when group slopes were equal or the individual group slopes when group slopes were unequal.

We used principal components analysis (PCA) to initially establish the presence of phenotypic divergence among fish spawning in different habitat types. Males ( $N = 317$ , 59–90 per habitat type) and females ( $N = 176$ , 32–59 per habitat type) were analyzed separately in MINITAB, version 11 (State College, Pennsylvania, USA) using the correlation matrix of phenotypic traits (males—adjusted BD and SN, and spawning color; females—adjusted BD, SN, and egg size, spawning color, and egg color). Significance of divergence among habitat types was assessed by comparison of 95% confidence intervals of principal component scores.

Because selection likely differs among traits, we followed our multivariate analysis with an investigation of phenotypic differences among habitat types for individual traits. Morphological traits and egg size were compared among habitat types with ANCOVA. When interaction effects between habitat type and body size were absent (slopes were equal), the interaction term was dropped from the model and differences in the adjusted mean trait values were tested for significance. When significant interaction effects were present between habitat type and body size, separate linear regressions for each habitat

**Table 1** Mean (SD) spawning habitat characteristics and color measures for 13 sockeye salmon populations in Lake Clark, Alaska

Sample site <sup>a</sup>	Depth (m)	Percent substrate <sup>b</sup>			Turbidity (NTU)	Spawning color		Egg color
		Fine	Moderate	Coarse		♂	♀	
GB1 Hatchet Point Beach	–	–	–	–	5.7 (1.5)	0.03	0.15	1.5
GB2 Little Lake Clark Beach	0.90	2	32	66	11.6 (1.3)	0.07	0.93	2.9
NGB1 Sucker Bay Lake	0.72	11	41	49	2.7 (1.0)	0	0	1.8
NGB2 Chi Point Beach	–	–	–	–	2.4 (0.2)	0	0	3.1
NGB3 Kijik Lake South Beach	0.48	16	80	4	0.7 (0.6)	0	0	3.4
Beach mean	0.70 (0.21)	10 (7)	51 (25)	40 (32)	4.6 (4.3)	0.02 (0.03)	0.22 (0.41)	2.5 (0.8)
GT1 Currant Creek	0.44	24	52	24	7.0	0	0.97	2.8
GT2 Lower Tlikakila River	0.38	24	56	20	6.2 (2.3)	0	0.63	2.3
GT3 Upper Tlikakila River	0.34	73	26	1	8.1	0.03	0.57	2.4
NGT1 Tazimina River	0.63	11	75	14	0.4 (<0.1)	0	0	1.3
NGT2 Kijik River	0.44	17	70	13	2.2 (2.4)	0	0	2.3
NGT3 Priest Rock Creek	0.51	66	32	2	1.6 (0.1)	0	0	1.3
Tributary mean	0.45 (0.10)	36 (27)	52 (20)	12 (9)	4.2 (3.2)	0.01 (0.01)	0.36 (0.42)	2.1 (0.6)
OT1 Lake Clark Outlet	–	–	–	–	2.9 (0.1)	0	0	2.0
OT2 Little Kijik River	0.47	10	87	3	0.6 (0.3)	0	0	2.5
Glacial mean	0.51 (0.26)	31 (30)	41 (15)	28 (27)	7.7 (2.4)	0.03 (0.03)	0.65 (0.33)	2.4 (0.6)
Non-glacial mean	0.54 (0.11)	22 (22)	64 (22)	14 (18)	1.7 (1.0)	0	0	2.2 (0.8)

Spawning color is the frequency of pink body color, egg color is the mean interval color rank from light (1) to dark (5) orange

Standard deviations within sites represent interannual variation

<sup>a</sup> *NGB* non-glacial beach, *GB* glacial beach, *NGT* non-glacial inlet tributary, *GT* glacial inlet tributary, *OT* outlet tributary (non-glacial)

<sup>b</sup> Fine, <4 mm; moderate, 4 to <64 mm; coarse, ≥64 mm



type were compared. Spawning and egg color were compared among habitat types by log-likelihood ratio ( $LR$ ), or  $G$ , statistics for contingency tables with exact significance calculated using a Monte Carlo method. Repeated spawning color scores were similarly compared between and within different observers. Within females, egg size and egg color were compared within habitat types with two-way ANOVA and spawning color and egg color compared among habitat types with one-way ANOVA. Uncorrected significance values are presented as results did not change with Bonferroni correction for multiple comparisons between habitat types and within traits and sexes (Rice 1989). Correlations between mean spawner phenotypes and spawning habitat parameters were assessed with simple regression. Two-tailed tests were used except where noted and to test predictions that specify direction of the effect.

Correlations between phenotypic divergence among populations, neutral genetic divergence among populations, and spawning habitat type were assessed with simple and partial Mantel tests (Smouse et al. 1986) in FSTAT version 1.2 (Goudet 1995) using the method described in Manly (1991). Pair-wise phenotypic divergence among populations was calculated as  $P_{ST}$  for BD, spawning color, and egg color. For BD, phenotypic variance components were estimated with one-way ANOVA in SPSS® and  $P_{ST}$  was calculated as

$$P_{ST} = \frac{\sigma_{GB}^2}{(\sigma_{GB}^2 + 2\sigma_{GW}^2)}$$

as in Raeymaekers et al. (2007) and Saint-Laurent et al. (2003). For spawning and egg color data, phenotypic variance components and  $P_{ST}$  were calculated using the ANOVA framework developed for categorical genetic data by Cockerham (1973). Traits were treated as haploid loci, phenotypic categories within traits were coded as alleles, and pair-wise  $P_{ST}$  was calculated as pair-wise  $F_{ST}$  in FSTAT version 1.2 (Goudet 1995) according to Weir and Cockerham (1984). Neutral genetic divergence among populations was calculated as pair-wise microsatellite  $F_{ST}$  (taken from Ramstad et al. 2004), computed in FSTAT according to Weir and Cockerham (1984), and based largely on the same individual fish sampled phenotypically for this study. Significance of differences between mean pair-wise  $P_{ST}$  and  $F_{ST}$  was assessed by comparison of 95% confidence intervals which were calculated by a non-parametric bootstrapping procedure where the observed pair-wise differentiation measures ( $P_{ST}$  or  $F_{ST}$ ) were sampled with replacement over 10,000 replicate data sets. Two spawning habitat matrices were defined as BT (beach or tributary) and GNG (glacial or non-glacial) and coded for populations spawning in similar (0) and different (1) habitat types.  $P_{ST}$  and  $F_{ST}$  were log transformed after zero values were reassigned a value of 0.0001 based on the smallest pair-wise  $F_{ST}$  detected in all population comparisons.

## Results

### Spawning habitat variation

We found significant variation in the physical parameters of the 13 spawning habitats sampled (Table 1). Beach and tributary habitats did not differ in mean turbidity ( $F_{1,9} = 0.30$ ,  $P = 0.87$ ). However, water depth was greater on average at beach than tributary spawning sites ( $F_{1,7} = 5.94$ ,  $P = 0.05$ ), and beaches tended to have coarser

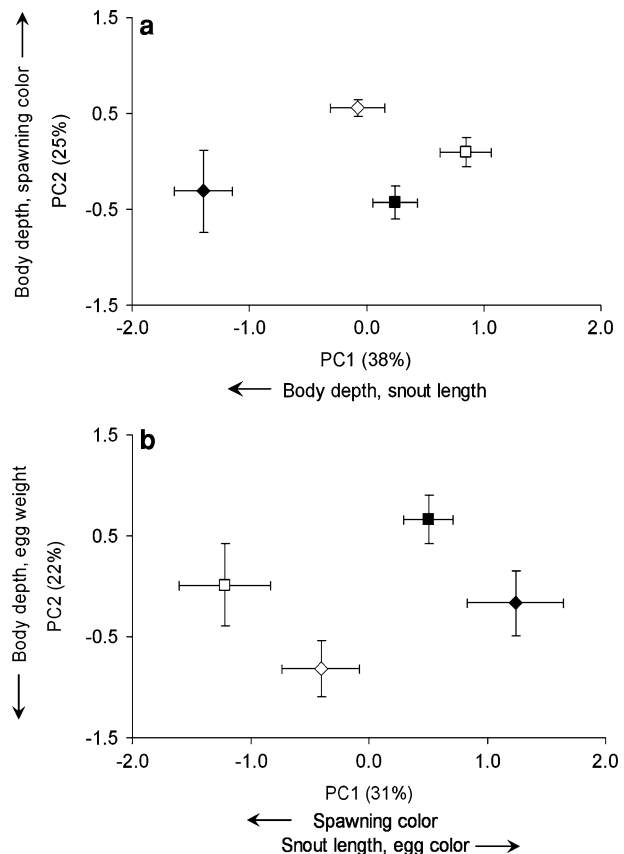
substrate than tributary spawning sites ( $F_{1,7} = 4.29$ ,  $P = 0.08$ ), though the difference was not statistically significant.

Turbidity during spawning was significantly higher in glacial than non-glacial habitats (outlet tributaries included;  $F_{1,11} = 41.64$ ,  $P < 0.001$ ; Table 1). This trend was apparent, but not statistically significant, among beach habitats ( $F_{1,3} = 8.00$ ,  $P = 0.07$ ) and highly significant among tributary habitats ( $F_{1,4} = 56.67$ ,  $P < 0.01$ ). Lack of data precluded testing for differences in depth and substrate size between glacial and non-glacial beaches. However, there was a tendency for glacial tributaries to be shallower ( $0.39 \pm 0.14$  m 95% CI) than non-glacial tributaries ( $0.52 \pm 0.23$  m;  $F_{1,4} = 4.86$ ,  $P = 0.09$ ), and no difference in substrate size composition between glacial and non-glacial tributaries ( $P \geq 0.43$ ).

### Comparison of phenotypic and habitat variation

Principal components analysis revealed that specific combinations of phenotypic traits are found in different habitat types for both males and females. Beach and tributary spawning males were differentiated by the first principal component (PC, not significant between non-glacial beaches and glacial tributaries, Fig. 2a). Males spawning on beaches tended to have deeper bodies (loading =  $-0.572$ ) and longer snouts ( $-0.672$ ) than males spawning in tributaries. Glacial and non-glacial spawning males were differentiated by the second PC

**Fig. 2** Principal components analysis of morphological (body depth, snout length, spawning color, and egg color) and life history (egg size) traits of male (a) and female (b) sockeye salmon in Lake Clark, Alaska. Points represent mean component scores by spawning habitat type coded as non-glacial beaches ( $\diamond$ ;  $N = 89$  males, 42 females), non-glacial inlet tributaries ( $\square$ ;  $N = 79$  males, 43 females), glacial beaches ( $\blacklozenge$ ;  $N = 59$  males, 32 females), and glacial inlet tributaries ( $\blacksquare$ ;  $N = 90$  males, 59 females). Percentages in parentheses indicate amount of variation explained by each principal component; bars represent 95% confidence intervals. Traits with the highest loadings are given with each axis; arrows indicate the direction of increasing trait values



(not significant for glacial beaches and non-glacial tributaries, Fig. 2a). Males spawning in glacial habitats tended to have shallower bodies (0.406) and lighter spawning color ( $-0.798$ ) than males spawning in non-glacial habitats. Spawning color scores were highly repeatable both within ( $LR_{4-5} = 1.3$ – $1.5$ ;  $P \geq 0.90$ ) and between observers ( $LR_4 = 1.3$ ;  $P = 0.90$ ).

Beach and tributary spawning females were differentiated by the second PC (not significant between glacial beaches and non-glacial tributaries; Fig. 2b). Females spawning on beaches tended to have deeper bodies (loading =  $-0.531$ ) and larger eggs ( $-0.638$ ) than females spawning in tributaries. Glacial and non-glacial spawning females were differentiated by the first PC (Fig. 2b). Females spawning in glacial habitats tended to have longer snouts (0.316), lighter spawning color (0.481), and darker egg color (0.524) than females spawning in non-glacial habitats.

### *Beach and tributary habitats*

**Morphology** Beach spawning fish had significantly deeper bodies (males = 9%, 17 mm; females = 6%, 7 mm) and longer snouts (males = 4%, 6 mm; females = up to 12%, 6 mm) than tributary spawning fish (Table 2). Male BD increased significantly with water depth ( $F_{1,8} = 3.80$ ,  $P_{(1)} = 0.04$ ,  $R^2 = 0.32$ ), but female BD and mean adjusted SN (both sexes) were independent of water depth ( $P > 0.17$ ).

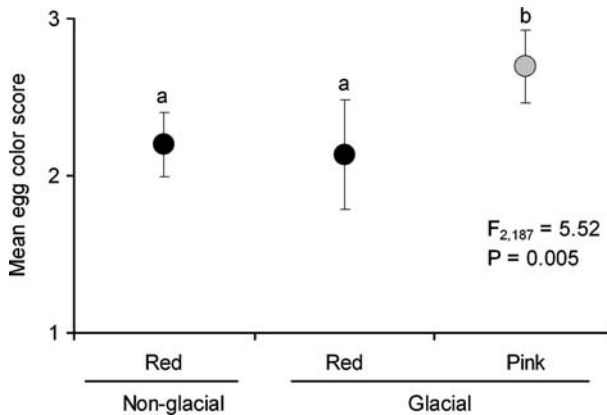
**Egg size** Egg size increased with body size in both beach and tributary fish. Beach spawners had 8% (0.008 g) larger eggs at all body sizes than tributary spawners (Table 2). Egg size and proportion of fine substrate were negatively correlated ( $F_{1,8} = 10.24$ ,  $P_{(1)} < 0.01$ ,  $R^2 = 0.56$ ).

**Spawning and egg color** Spawning color was similar between males ( $LR_1 = 1.3$ ,  $P = 0.34$ ), but differed significantly between females ( $LR_2 = 9.3$ ,  $P < 0.01$ ) spawning in beach and tributary habitats due to a higher frequency of red females in beach than tributary populations (Table 1). Female body color was not correlated with either water depth or substrate size ( $P \geq 0.82$ ). Beach spawning females had significantly darker eggs than tributary spawning females ( $LR_4 = 12.5$ ,  $P = 0.01$ ), but this was due only to the darker eggs of the NGB3 population (Table 1). Egg color was independent of egg size within habitat types ( $F_{4,309} = 1.0$ ,  $P = 0.43$ ) and was not correlated with either water

**Table 2** Difference in body depth (BD, mm), snout length (SN, mm) and egg size (g) between Lake Clark sockeye salmon spawning in beach and tributary habitats

Sex	Variable	Beach	Tributary	<i>N</i>	<i>F</i>	Adjusted body size (mm)
M	BD	204.0 (202.4–205.6)	186.8 (185.3–188.3)	190, 190	206.5**	519
	SN	107.1 (106.1–108.2)	102.6 (101.6–103.6)	189, 189	37.0**	519
F	BD	138.0 (136.6–139.4)	130.7 (129.7–131.8)	190, 200	71.7**	499
	SN	NA	NA	189, 200	8.1**	NA
	Egg size	0.114 (0.112–0.117)	0.106 (0.105–0.107)	104, 184	37.1**	505

Significance (\*  $P < 0.05$ , \*\*  $P < 0.01$ ) was tested by analysis of covariance with  $\log_e$  transformed body length as the covariate and habitat type as a factor. Where trait size increased similarly with body size between groups, traits were adjusted to a common body size within groups and group means are given (95% confidence intervals). When trait size differences between groups varied with body size, results represent significance of interaction effects between body and trait size



**Fig. 3** Mean egg color score for female sockeye salmon with red (*black points*) and pink (*grey point*) spawning color in glacial and non-glacial habitats of Lake Clark, Alaska. Bars represent 95% confidence intervals, egg color scores are the closest matching of plates one (lightest) through five (darkest) of the Hoffman La-Roche color card for salmonids

depth or substrate size ( $P > 0.43$ ). Female body color and egg color were negatively correlated ( $F_{1,188} = 11.0$ ,  $P < 0.01$ ). Eggs were lighter in color among red females (mean egg color score glacial:  $2.13 \pm 0.35$  95% CI; non-glacial:  $2.20 \pm 0.21$ ) than pink females ( $2.69 \pm 0.23$ ;  $F_{2,187} = 5.52$ ,  $P < 0.01$ ; Fig. 3).

To summarize, sockeye salmon populations spawning on Lake Clark beaches generally had deeper bodies, longer snouts, larger eggs, and higher frequencies of red females than sockeye salmon populations spawning in Lake Clark tributaries. Spawning site water depth was positively correlated with male body depth and proportion of fine substrate was negatively correlated with egg size. Correlations with measured habitat characteristics could not account for differences in SN, spawning color, or egg color between beach and tributary populations.

#### *Glacial and non-glacial habitats*

**Morphology** Glacial beach spawning fish had similar BD and greater SN (males = up to 6%, 7 mm; females = 3%, 2 mm) than non-glacial beach spawning fish (Table 3). Males spawning in glacial tributaries had shallower bodies (4%, 9 mm) and longer snouts (5%, 5 mm) than males spawning in non-glacial tributaries. Females spawning in glacial tributaries had similar BD but greater SN (up to 4%, 2 mm) than females spawning in non-glacial tributaries (Table 3). Mean BD ( $P_{(1)} > 0.39$ ) and adjusted SN ( $P_{(1)} > 0.14$ ) were not correlated with turbidity in either males or females overall, but SN was correlated with turbidity among tributary spawning males (Fig. 4a).

**Egg size** Egg size did not differ between glacial and non-glacial beach spawning populations (Table 3). However, the relationship between egg size and body size differed between glacial and non-glacial tributary populations (Table 3). Mean egg size was greater in glacial than non-glacial tributary populations at hypural lengths below 510 mm and vice versa at larger body sizes. Adjusted mean egg size and turbidity were not correlated ( $P_{(1)} = 0.48$ ).

**Table 3** Difference in body depth, snout length, and egg size between Lake Clark sockeye salmon spawning in glacial and non-glacial habitats

Sex	Spawning habitat	Variable	Glacial	Non-glacial	<i>N</i>	<i>F</i>	Adjusted body size (mm)
M	Beach	BD	203.6 (201.1–206.0)	202.8 (200.8–204.8)	80, 110	0.3	517
		SN	NA	NA	80, 109	5.6*	NA
	Tributary	BD	184.2 (182.0–186.4)	192.7 (190.0–195.4)	110, 80	19.3**	521
		SN	105.1 (103.9–106.4)	100.5 (98.9–102.1)	110, 79	17.7**	521
F	Beach	BD	138.1 (136.2–140.1)	136.7 (135.1–138.4)	80, 110	1.0	497
		SN	71.6 (70.3–72.9)	69.3 (68.2–70.5)	80, 109	7.3**	497
		Egg size	0.114 (0.111–0.117)	0.113 (0.110–0.116)	50, 54	0.2	503
	Tributary	BD	130.6 (129.0–132.2)	132.4 (130.6–134.3)	110, 90	2.0	501
		SN	NA	NA	110, 90	5.2*	NA
		Egg size	NA	NA	104, 80	20.4**	NA

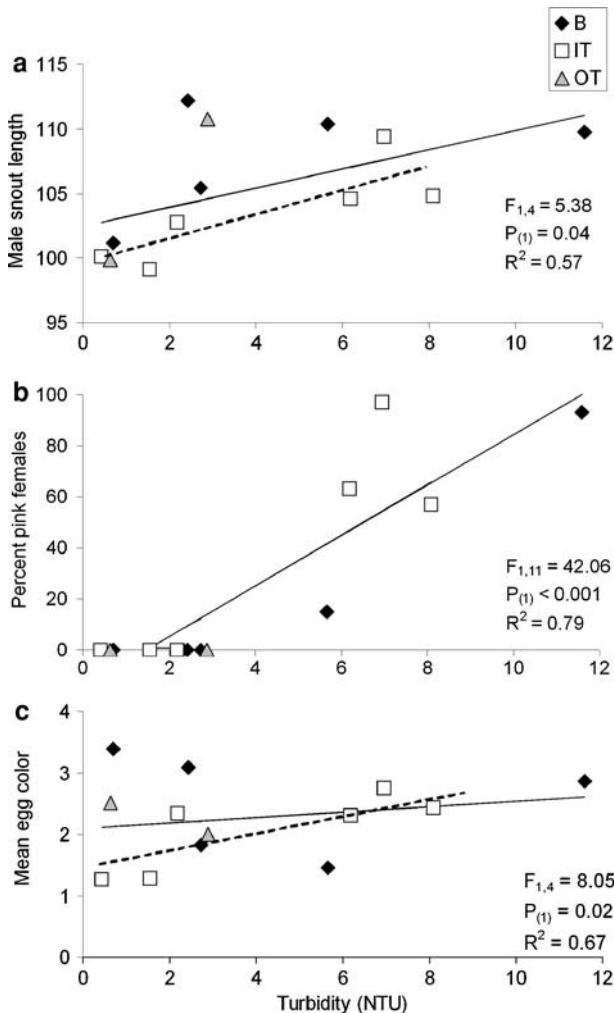
See footnote of Table 2

**Spawning and egg color** Males were primarily red, with no difference in color between glacial and non-glacial habitats (beach:  $LR_1 = 5.7$ ,  $P = 0.06$ ; tributary:  $LR_1 = 1.3$ ,  $P = 1.0$ ; Table 1). In contrast, female body color differed significantly between glacial and non-glacial habitats (beach:  $LR_1 = 77.1$ ,  $P < 0.01$ ; tributary:  $LR_2 = 132.2$ ,  $P < 0.01$ ), with primarily pink females in glacial (beach = 57%; tributaries = 72%) and only red females in non-glacial habitats (Table 1). Egg color did not differ between glacial and non-glacial beach populations ( $LR_3 = 5.1$ ,  $P = 0.18$ ). However, glacial tributary populations had significantly darker eggs than non-glacial tributary populations ( $LR_4 = 45.3$ ,  $P < 0.01$ ; Table 1). Turbidity was positively correlated with pink female body color among all populations surveyed (Fig. 4b) and with mean egg color among tributary populations (Fig. 4c).

In summary, sockeye salmon populations spawning in glacial habitats had longer snouts and higher frequencies of pink females than sockeye salmon populations spawning in non-glacial habitats. In addition, populations spawning in glacial tributaries had shallower bodied males, different egg size allometries, and darker colored eggs than populations spawning in non-glacial tributaries. Spawning site turbidity was correlated with male SN (tributary habitats only), female body color, and egg color (tributary habitats only). Differences in water turbidity could not explain differences observed in body depth or egg size.

#### Comparison of phenotypic and neutral genetic divergence

Mean  $P_{ST}$  was not correlated with mean  $F_{ST}$  for BD (males:  $P = 0.89$ ; females:  $P = 0.93$ ) or body color (males:  $P = 0.19$ ; females:  $P = 0.06$ ), but was correlated with  $F_{ST}$  for egg color ( $P < 0.01$ ). Body depth  $P_{ST}$  was correlated with the BT habitat matrix for males ( $P < 0.01$ ) but not females ( $P = 0.06$ ) and was not correlated with the GNG habitat matrix for either sex ( $P > 0.30$  males and females). In contrast, spawning color was not significantly correlated with the BT habitat matrix ( $P \geq 0.18$  males and females), but was correlated with the GNG habitat matrix ( $P < 0.01$  males and females), which explained very little of the spawning color variation among males ( $R^2 < 0.01$ ) but the majority of

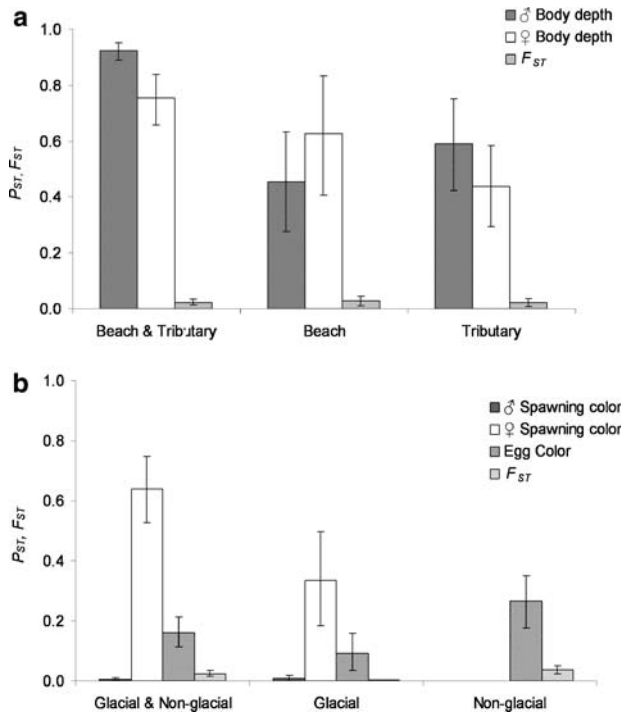


**Fig. 4** Correlation between turbidity during spawning and **a** mean male snout length (adjusted to common hypural length of 519 mm), **b** percent of female sockeye salmon with pink body color, and **c** mean egg color (by visual color score) by habitat type. Dashed lines and corresponding statistics are for inlet tributary populations only

variation among females ( $R^2 = 0.63$ ). Egg color was not correlated with either habitat matrix ( $P \geq 0.23$ ), regardless of whether we first removed the variation explained by  $F_{ST}$ .

Body depth  $P_{ST}$  exceeded  $F_{ST}$  in comparisons between and within beach and tributary habitats for both males and females (Fig. 5a). Mean BD  $P_{ST}$  was greater between beach and tributary populations than among populations of similar habitat types ( $P < 0.05$  beach and tributary populations males, tributary populations females). The greatest BD  $P_{ST}$  observed was between beach and tributary spawning males.

Male and female spawning color  $P_{ST}$  was undefined among non-glacial populations because all fish were red. Male spawning color  $P_{ST}$  was less than  $F_{ST}$  in comparisons of glacial and non-glacial populations ( $P < 0.05$ ), but similar to  $F_{ST}$  in comparisons among



**Fig. 5** Comparison of mean pair-wise  $P_{ST}$  and  $F_{ST}$  of sockeye salmon spawning within and between **a** beach and tributary and **b** glacial and non-glacial habitats of Lake Clark, Alaska. Bars represent 95% confidence intervals

glacial populations (Fig. 5b). Female spawning color  $P_{ST}$  exceeded  $F_{ST}$  in comparisons between glacial and non-glacial populations and among glacial populations. In females, spawning color  $P_{ST}$  was significantly greater in comparisons between glacial and non-glacial populations than in comparisons among glacial populations, but the two  $P_{ST}$  values were similar in males. The greatest spawning color  $P_{ST}$  observed was between glacial and non-glacial spawning females. Egg color  $P_{ST}$  significantly exceeded  $F_{ST}$  in all comparisons and was greater among non-glacial populations than among glacial populations ( $P < 0.05$ ; Fig. 5b).

## Discussion

Our data suggest that Lake Clark sockeye salmon are locally adapted to glacial, beach, and tributary spawning habitats. Phenotypic variation among populations is correlated with differences in spawning habitat and likely due to differences in the extent and nature of both natural and sexual selection among spawning habitat types. In addition, phenotypic divergence among populations is independent of neutral genetic divergence among populations, and, for most traits, greater than expected based on genetic drift alone.

Phenotypic plasticity is an unlikely source of phenotypic differences among populations because Lake Clark sockeye salmon spend most of their lives in a common environment (as discussed in the 'Introduction'). While environmental effects may differentially affect

phenotypes during incubation or on a microhabitat scale, there is otherwise little opportunity for Lake Clark sockeye salmon populations to experience different environments. In addition, the phenotypic traits we measured are fully developed prior to fish arriving at their spawning habitats and have been shown to have moderate to high heritabilities in a number of salmonid populations and environmental settings (morphology—Funk et al. 2005; Keeley et al. 2007, egg size—Gall and Neira 2004; but see Funk et al. 2005, carotenoid conversion—Craig and Foote 2001).

Estimates of  $P_{ST}$  based on few populations have low precision and are potentially biased (O'Hara and Merila 2005; Goudet and Buchi 2006). However, this is expected to have little effect on our overall results. The problem of low precision is less acute for comparisons among populations that are poorly differentiated at neutral genetic traits ( $F_{ST} < 0.02$ , Goudet and Buchi 2006), such as Lake Clark sockeye salmon. While our  $P_{ST}$  confidence intervals are wide, our mean  $P_{ST}$  estimates are well above the 95%  $F_{ST}$  confidence interval for most traits and comparisons because microsatellite  $F_{ST}$  is low. This suggests our results are robust to changes in assumptions about the heritability and additive genetic variance levels for the phenotypic traits considered (Saether et al. 2007).  $P_{ST}$  will be biased downward when estimated among few populations that are strongly divergent phenotypically (O'Hara and Merila 2005; Goudet and Buchi 2006). This effect, however, would make our  $P_{ST}$  estimates conservative, and suggests that our  $P_{ST} > F_{ST}$  results are not a statistical artefact. In contrast, mean  $P_{ST}$  was less than  $F_{ST}$  for a single trait (male spawning color) compared among glacial and non-glacial habitats. This could be due to a downward bias in the  $P_{ST}$  estimate due to non-additive genetic and environmental effects (Goudet and Buchi 2006; Whitlock 2008) or the homogenizing effects of selection.

#### Lake Clark founder event

Most populations included in this study exhibit a common genetic bottleneck signal due to a founder event associated with the colonization of Lake Clark by sockeye salmon (Ramstad et al. 2004). However, the bottleneck is mild (decrease in microsatellite heterozygosity of <5%) and old (>100 sockeye salmon generations, Ramstad et al. 2004) and should not affect the ability of these populations to adapt locally. If the Lake Clark founder event had a lasting effect on quantitative trait variation, we should observe phenotypic divergence between those populations that were commonly founded and those that were not (OT1-Lake Clark Outlet and NGT1-Tazimina River). We found no evidence of such a pattern. Thus, genetic drift due to the Lake Clark founder event cannot explain the patterns of phenotypic divergence observed among populations in this study.

Commonly founded populations have provided some of the best evidence of rapid adaptive divergence within species because phenotypic divergence due to selection must have arisen since populations became established (Hendry et al. 2000b; Quinn et al. 2000; Stockwell et al. 2003; Barluenga et al. 2006). These studies suggest that local adaptation can occur quickly (e.g., 13 generations in Lake Washington sockeye salmon) and be present among populations that exhibit little neutral genetic divergence (Hendry et al. 2000b; Stockwell et al. 2003). Local adaptation of sockeye salmon to glacial habitats, which are young and ephemeral, suggests this adaptation is also recent.

#### Beach and tributary habitats

Our data support the substantial body of literature suggesting local adaptation of sockeye salmon to beach and tributary habitats (reviewed in Hilborn et al. 2003). We found similar



patterns of phenotypic divergence (deeper bodies and larger eggs among beach than tributary spawners) among Lake Clark sockeye salmon as in other lake systems (Blair et al. 1993; Quinn et al. 1995, 2001; Hendry et al. 2000b). This parallel pattern of divergence cannot be due to random genetic drift and provides indirect evidence that such differences are adaptive. Additionally, divergence in male and female body depth is greater between than within beach and tributary habitat types and significantly greater than can be explained by neutral genetic drift alone. Taken together, these data support the view that sockeye salmon in Lake Clark have adapted locally to beach and tributary spawning habitats.

### Glacial habitats

This study provides the first evidence of local adaptation of salmon to glacial spawning habitats. Glacial habitats have elevated turbidities, less stable flow regimes, finer substrates, and lower temperatures than non-glacial habitats (Stanford and Ward 1992; Murphy et al. 1997). Sockeye salmon spawning in glacial habitats of Lake Clark generally had longer snouts, lighter spawning color, and darker egg color than fish spawning in non-glacial habitats of Lake Clark. Male snout length, female spawning color, and egg color were all significantly correlated with spawning habitat turbidity. Spawning color  $P_{ST}$  was greater than  $F_{ST}$  for females and less than  $F_{ST}$  for males in comparisons between glacial and non-glacial habitats. This suggests that selection may favor different phenotypes among females spawning in glacial (pink spawning color) and non-glacial habitats (red spawning color) and similar phenotypes among males (red spawning color) spawning in both types of habitat.

### *Significance of snout length*

Sockeye salmon develop elongated snouts with large, sharp teeth during their homeward spawning migration (Hamon and Foote 2000; Witten and Hall 2002). Snouts are more exaggerated in males than females, but both sexes use their snouts as intrasexual weapons (Darwin 1871; Quinn and Foote 1994) and snout length is positively correlated with spawning density (Fleming and Gross 1989). It is unlikely, however, that fish spawning in glacial habitats have longer snouts than fish spawning in non-glacial habitats due to increased breeding competition. We have no reason to believe that spawning density is greater in glacial than non-glacial populations of our study. Further, redd size tends to decrease and spawning color intensity increase with spawning competition (Mathisen 1962; Fleming and Gross 1989), but sockeye salmon construct larger redds (Lorenz and Eiler 1989) and have lighter spawning color in glacial than non-glacial habitats. These observations suggest that fish spawning in glacial habitats may perceive low spawning density because their ability to see competitors is reduced. Secchi depths near Little Lake Clark Beach, the most turbid of all glacial spawning habitats we sampled, are less than 1 m during spawning underscoring the poor visibility in glacial spawning habitats (Wilkens, unpublished data). Low visibility in glacial habitats may reduce the ability of individuals to avoid aggressive encounters through visual displays of body size and status and promote increased investment in the development of weapons. Reduced body depth among males spawning in glacial tributaries (relative to non-glacial tributaries) is consistent with this view, as is lighter spawning color among glacial spawning females.

### *Significance of spawning and egg color*

Sockeye salmon obtain carotenoid pigments through their diet while at sea, sequester them in their flesh, and then transfer them to their skin and eggs in preparation for spawning (Crozier 1970; Goodwin 1984). The resulting red spawning color is important for mate recognition and mate choice (Craig and Foote 2001; Foote et al. 2004) and appears to indicate social status for both sexes (Fleming and Gross 1989, 1994). Sockeye salmon have a keen ability to see red while spawning (Beatty 1966; Novales-Flamarique 2000), and males preferentially mate with the reddest female available (Foote et al. 2004). Carotenoid-based sexual signals are thought to be honest signals of the fitness and status of individuals (Olson and Owens 1998). This is because carotenoids pigments are relatively rare in nature but are important for protecting tissues from oxidative damage and regulating immune response (Olson and Owens 1998; Blount et al. 2000; but see Hartley and Kennedy 2004). Animals cannot synthesize carotenoids *de novo* and must acquire them through diet. It is also important to provision eggs with carotenoids to improve the immune response and survival of young (Blount et al. 2000, 2002; Tyndale et al. 2008). Many organisms concentrate carotenoids in their eggs at the expense of their own body color and, potentially, effective immune response (Green 1965; Skarstein and Folstad 1996; Royle et al. 2003; Nordeide et al. 2006; Baeta et al. 2008).

In Lake Clark, sockeye salmon spawning in glacial habitats have lighter spawning color than those spawning in non-glacial habitats and this difference is strongly pronounced in females. Why might we observe such a pattern? Differences in diet (Craig and Foote 2001; Miller et al. 2007) do not likely cause spawning color differences between populations because sockeye salmon source carotenoids from their marine diet and we have no reason to believe that glacial and non-glacial spawning fish or sexes are segregated while at sea. Predation and sexual selection for pink spawning color are also unlikely causes of spawning color differences because there is no evidence of lighter spawning color among sockeye salmon spawning in environments of high chromatic contrast and intense brown bear (*Ursus arctos*) predation (e.g., Hansen Creek, Alaska, Ramstad, personal observation; Ruggerone et al. 2000) and males appear to prefer red color of an intensity beyond that observed in spawning females (Foote et al. 2004).

The most parsimonious explanation for differences in spawning color between glacial and non-glacial spawning populations is relaxed sexual selection for red spawning color in glacial habitats, coupled with a trade-off in body and egg color in females. In non-glacial habitats, females may develop red body color at the expense of their eggs because red is an effective signal allowing semelparous females to advertise to potential mates during their short breeding lifespan. In glacial habitats, females may provision their eggs with carotenoids at the expense of developing red spawning color because red is an ineffective signal. This pattern has not been observed previously because sockeye salmon are typically studied in clear-water habitats with the assumption that sexual selection is constant. Red body color is a common signal in freshwater fishes but becomes ineffective and is often lost when increased turbidity renders it invisible [e.g., haplochromine cichlids (*Haplochromis nyererei*), Seehausen et al. 1997] or when spawning habitats do not contrast well with red color [e.g., tannin stained waters, three-spine stickleback (*Gasterosteus aculeatus*), Boughman 2001; bluefin killifish (*Lucania goodie*), Fuller 2002].

Similar spawning color selection would likely affect males and females differently because males have no alternative use for their carotenoids while females must partition their carotenoids between themselves and their young (Craig and Foote 2001; Foote et al. 2004; Nordeide et al. 2006). Our data are consistent with this apparent trade-off between

body and egg color in female sockeye salmon (Crozier 1970; Craig and Foote 2001; Foote et al. 2004). Thus, spawning color differences between glacial and non-glacial spawning females could be a by-product of selection on egg carotenoid concentration in glacial habitats or vice versa. Glacial habitats tend to have fine substrate and poor inter-gravel water circulation which could expose eggs and pre-emergent fry in glacial redds to lower dissolved oxygen concentrations and increased levels of free radicals. These conditions could result in selection for higher carotenoid concentration in eggs and a differential partitioning of carotenoids between body and egg color in fish spawning in glacial and non-glacial habitats.

## Conclusions

Phenotypic divergence among spawning populations of Lake Clark sockeye salmon appears to be the result of local adaptation. The data provide additional evidence of beach and tributary ecotypes already reported in the literature, as well as the first evidence of a glacial ecotype of salmon. Our observations suggest that local adaptation of sockeye salmon to glacial spawning habitats involves reduced intensity of visual signals and increased weapon size. Because visual displays are not effective in avoiding aggressive encounters in highly turbid glacial habitats, individuals must arm themselves with effective weapons. Future studies should assess the existence of parallel patterns of phenotypic divergence in additional freshwater systems, undertake controlled common garden experiments to estimate  $Q_{ST}$  between glacial and non-glacial spawning populations, and directly measure the effects of environmental differences in the wild through reciprocal translocation experiments (Whitlock 2008). The presence of a glacial ecotype of sockeye salmon suggests that the excellent colonizing ability of this species may be due in part to an ability to adapt quickly to highly unstable, geologically young habitats.

**Acknowledgments** Funding was provided by the US Fish and Wildlife Service, the National Park Service, the US Geological Survey, and the National Science Foundation Training within Environmental Biology Program (T-WEB, University of Montana). The National Park Service also provided critical logistical field support. Additional funding from the Philanthropic Educational Organization supported KR during the writing of this manuscript. Thanks to J. Allgeier, R. Corcoran, P. Hernandez, C. Kasemodel, K. Kilcoyne, P. Knuckles, D. Oswald, T. Rinaldi, D. Rugerillo, H. Rugerillo, C. Saunders, M. Stafford, D. Ucitel, and J. Zutz for assistance with field work, T. Brabets for temperature data, A. Wilkens for turbidity and secchi depth data, and E. Benolkin for measuring egg color and size. Thanks to D. Young for the many ways he helped make this research successful. Thanks also to H. Sweep for Fig. 1, and L. Eby, D. Emlen, C. Foote, S. Mills, J. Reynolds, A. Sheldon, B. Weir, J. Porritt and C. Angels for helpful discussions and reviews of this manuscript. This paper was significantly improved by comments from three anonymous reviewers.

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